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Observations on the Wound Repair Processes in the Freshwater Crayfish *Procambarus* sp.

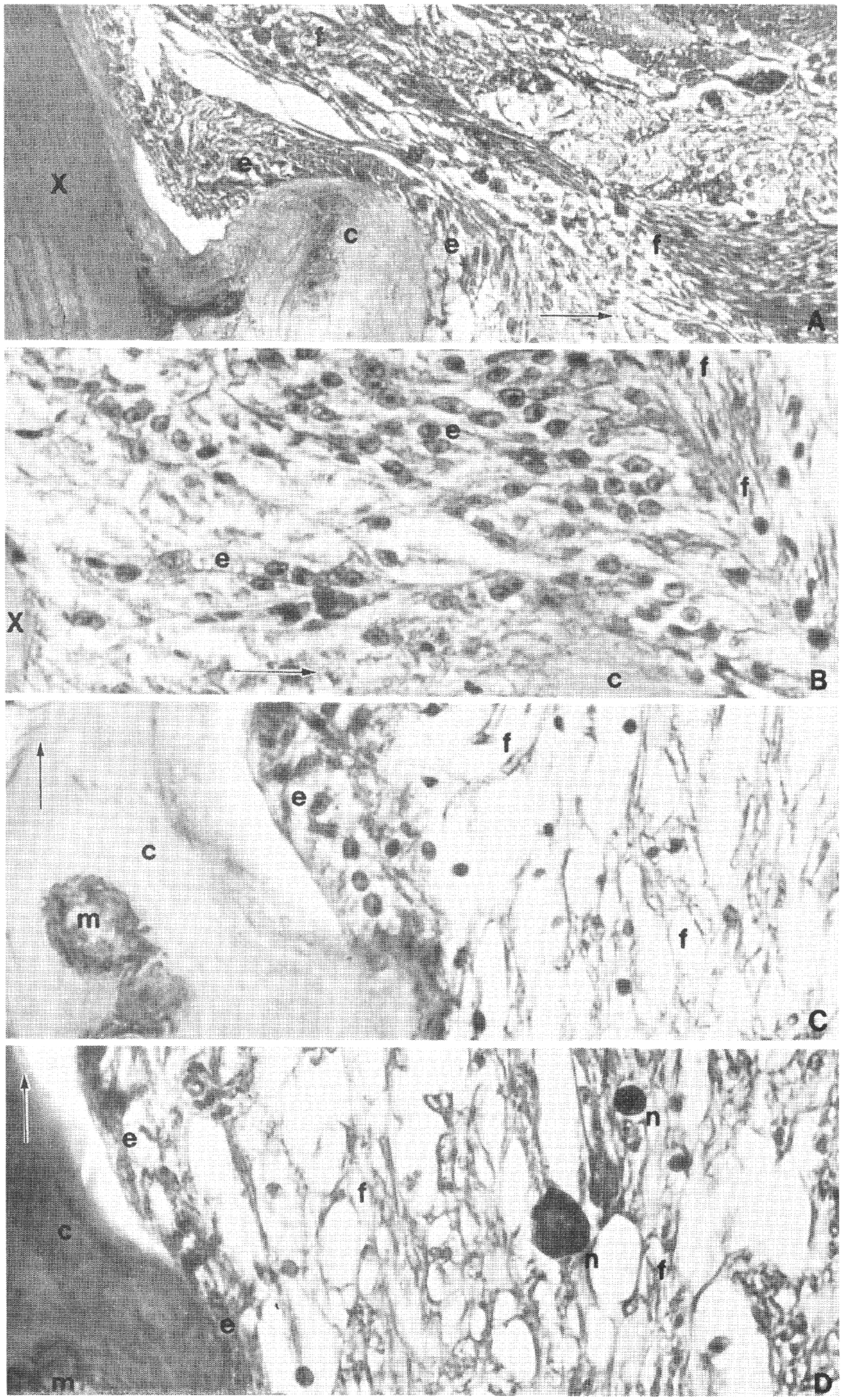
The wound repair processes of freshwater crayfish after injury by two different experimental procedures have been reported (L. H. Bretschneider, *Archineer. Zool.* **3,** 22–26, 1938; J. N. Dent and J. F. Fitzpatrick, Jr., *Ass. Southeast. Biol.* Bull. **10,** 26–27, 1963). Recently, the repair processes of penaeid shrimp after wounding with the Petersen disk tag (R. A. Neal, *U.S. Fish Wildlife Serv. Circ.* **295,** 1–15, 1968) were documented, both grossly (C. T. Fontaine, *J. Invertebr. Pathol.* **18,** 301–303, 1971) and histologically (C. T. Fontaine and D. V. Lightner, *J. Invertebr. Pathol.* **22,** 23–33, 1973). The purpose of the study reported here was to compare the processes of wound repair in the crayfish, *Procambarus* sp., after wounding with the Petersen disk tag to that which occurs in the penaeid shrimp.

The crayfish used in this study were collected from freshwater drainage ditches near Hitchcock, Texas, and were identified as members of the genus *Procambarus* (R. W. Pennak, "Freshwater Invertebrates of the United States," Ronald Press, New York, 1953). Wounding was accomplished by inserting the stainless steel tag pin through the abdomen between the second and third abdominal segments; no antibiotics were used. A total of 32 crayfish were wounded and held in dechlorinated tap water at 20°–29°C. Two crayfish were removed from the treated population at 6, 12, 24, 48, 72, and 96 hr; thereafter, samples were taken at 8, 12, 16, 20, 24, 28, 32, 36, and 40 days after wounding. The tissue immediately surrounding the tag pin was excised and routinely processed for paraffin embedding. Tissue sections 6–8 μm in thickness were stained with either Harris' hematoxylin, periodic acid–Schiff, Mallory's triple stain, or chlorozol black e (R. D. Lillie, "Histopathological Technic and Practical Histochemistry." 3rd. ed., McGraw-Hill, New York).

Grossly, the cuticle immediately adjacent to the entrance and exit wounds of the tag pin became blackened at 48 hr postwounding and remained blackened throughout the study. Unlike penaeid shrimp, the crayfish lost the cuticular tube formed around the tag pin during molts that occurred at 10 days after wounding.

The first histopathological event observed through 96 hr after wounding was the migration of hemocytes into the area of the wound. Plating or encapsulating of the tag pin, and formation of a dense black membrane then ensued. The black pigment presumably was melanin (T. Unestam and J. Nylund, *J. Invertebr. Pathol.* **19,** 94–106, 1972). The epidermis then migrated into the wound (Fig. 1A, B) basal to the black membrane at 8 days and secreted a cuticle between the apical ends of the epidermal cells and the black membrane. This black scablike membrane was then sloughed into the lumen of the wound. At 49 days postwounding the epidermis had migrated the length of the pin and the cuticular sheath was continuous throughout the wound (Fig. 1C, D). Basal to the epidermis along the wound channel, extensive scar tissue was formed that was composed of hemocytes, fibrocytes, and numerous collagenous fibers. Interspersed throughout this fibrous tissue were black nodules marking the foci of hemocytic encapsulations (Fig. 1D) that were probably formed in response to bacteria or other foreign material introduced during wounding.

The wound repair processes of the crayfish and shrimp are comparable with two notable exceptions. The crayfish, unlike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shrimp, lost the cuticular sheath formed around the tag pin during molting. Histologically, there was no connective tissue formed in the crayfish between the migrating epidermis and the new cuticle along the wound channel. A probable explanation of these differences is that rigidity of the crayfish exoskeleton reduced the movement of the pin in the wound. Hence, movement of the pin did not cause the cuticular sheath to become detached from the apical surface of the epidermis. Consequently, a new cuticle was produced during each molt cycle. The second exception was that the development of scar tissue basal to the epidermis in the crayfish was not organized to the extent seen in shrimp and had a loose alveolar structure.

C. T. FONTAINE

Galveston Laboratory
Gulf Coastal Fisheries Center
National Marine Fisheries Service
Galveston, Texas 77550

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This is contribution No. 385 from this laboratory.

FIG. 1A. Histopathological response near opening of wound channel at 8 days postwounding. X = exoskeleton, c = new cuticle, e = migrating epidermis, f = fibrous scar tissue, arrow indicates direction of wound channel. 260 ×, Mallory's triple stain. B. Migration of epidermal epithelial cells into and along the pin wound channel 12 days postwounding. X = exoskeleton, c = new cuticle, e = epithelial cells, f = fibrous scar tissue, arrow indicates direction of wound channel. 420 ×, Harris' hematoxylin and eosin. C. Wound repair processes near opening of wound channel at 40 days postwounding. c = new cuticular sheath, m = melanized areas, e = epidermis, f = vacuolated fibrous scar tissue, arrow indicates direction of wound channel. 420 ×, periodic acid–Schiff. D. Wound-repair processes deep along wound channel at 40 days postwounding. c = new cuticular sheath, m = melanized area, e = epidermis, f = vacuolated fibrous scar tissue, n = black nodules of encapsulation, arrow indicates direction of wound channel. 420 ×, Mallory's triple stain.